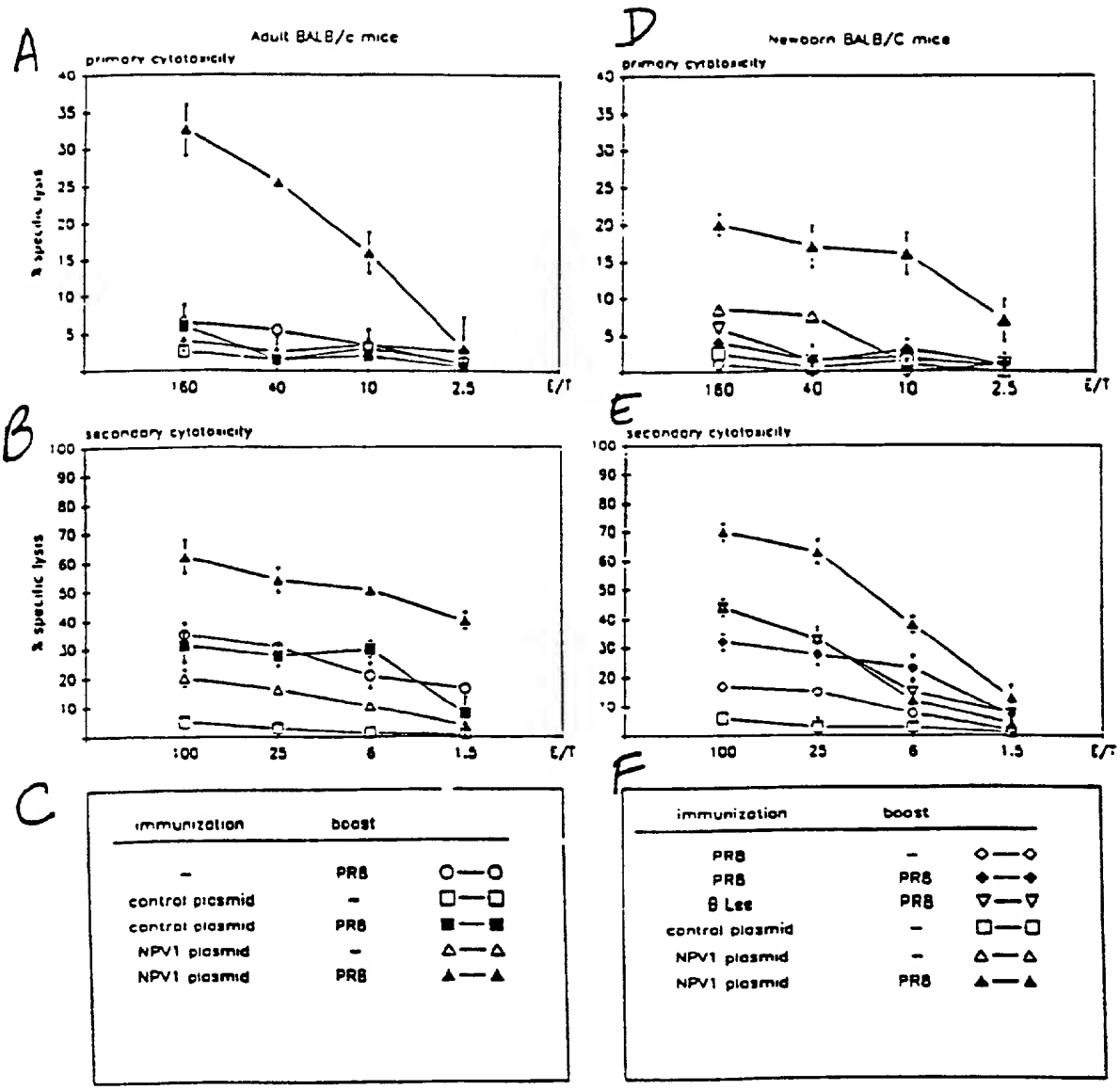
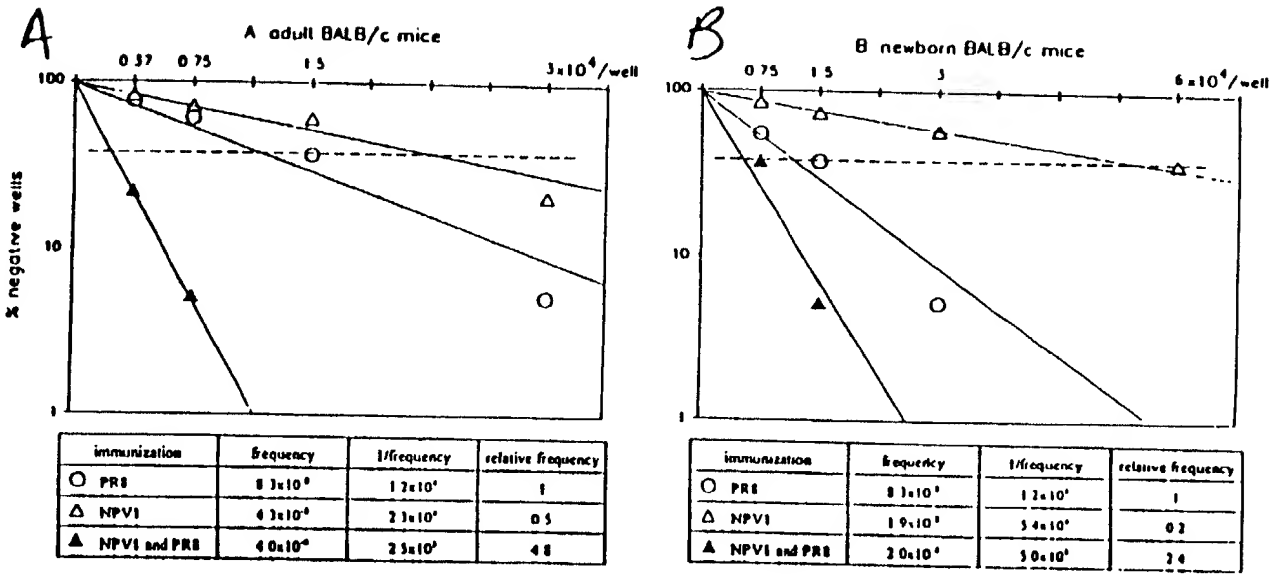

A
Adult BALB/c mice
primary cytotoxicity
% specific lysis
160
40
10
2.5
E/T
B
secondary cytotoxicity
% specific lysis
100
25
6
1.5
E/T
C
immunization
boost
– PRB
control plasmid –
control plasmid PRB
NPV1 plasmid –
NPV1 plasmid PRB
D
Newborn BALB/C mice
primary cytotoxicity
E
secondary cytotoxicity
F
immunization
boost
PRB –
PRB PRB
B Lee PRB
control plasmid –
NPV1 plasmid –
NPV1 plasmid PRB

Fig. 1

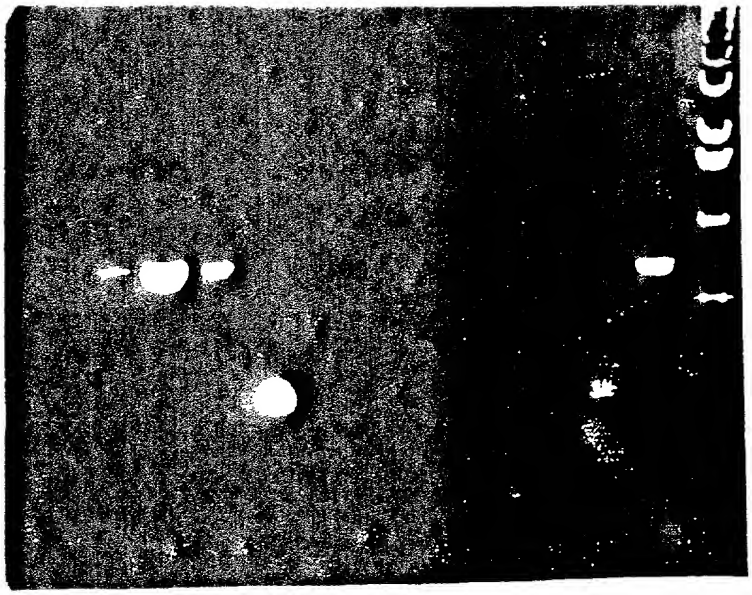


immunization	frequency	1/frequency	relative frequency
○ PR8	8 3x10⁻⁶	1 2x10⁵	1
△ NPV1	4 3x10⁻⁶	2 3x10⁵	0 5
▲ NPV1 and PR8	4 0x10⁻⁷	2 5x10⁶	4 8

immunization	frequency	1/frequency	relative frequency
○ PR8	8 3x10⁻⁶	1 2x10⁵	1
△ NPV1	1 9x10⁻⁶	5 4x10⁵	0 2
▲ NPV1 and PR8	2 0x10⁻⁷	5 0x10⁶	2 4

Fig. 2

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13.



1- 4. Adult: right anterior tibial muscle.
5. Adult: left anterior tibial muscle.
6-10. Newborn: right gluteal muscle.
11. Newborn: left gluteal muscle.
12. NPV1 plasmid.
13. DNA ladder.

Fig. 3

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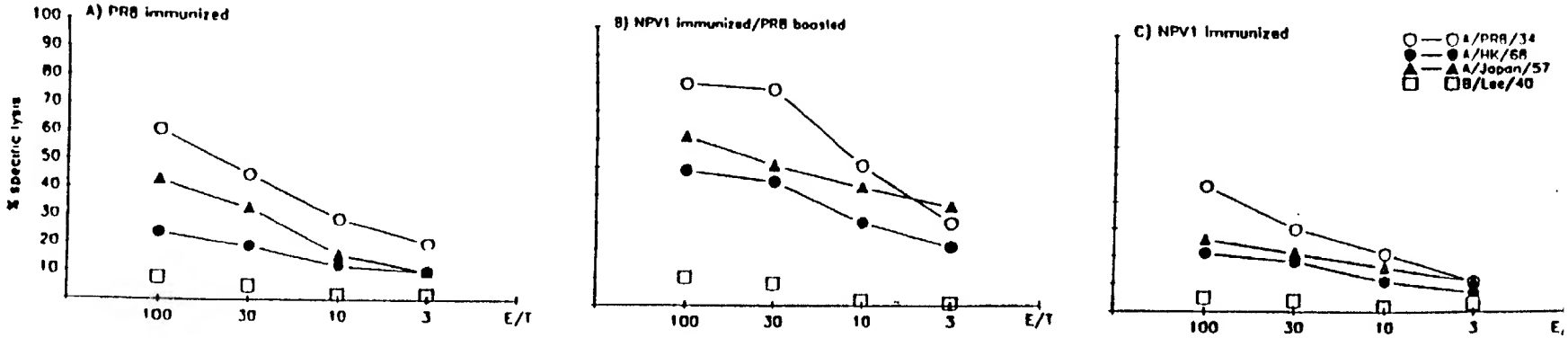


FIG 1

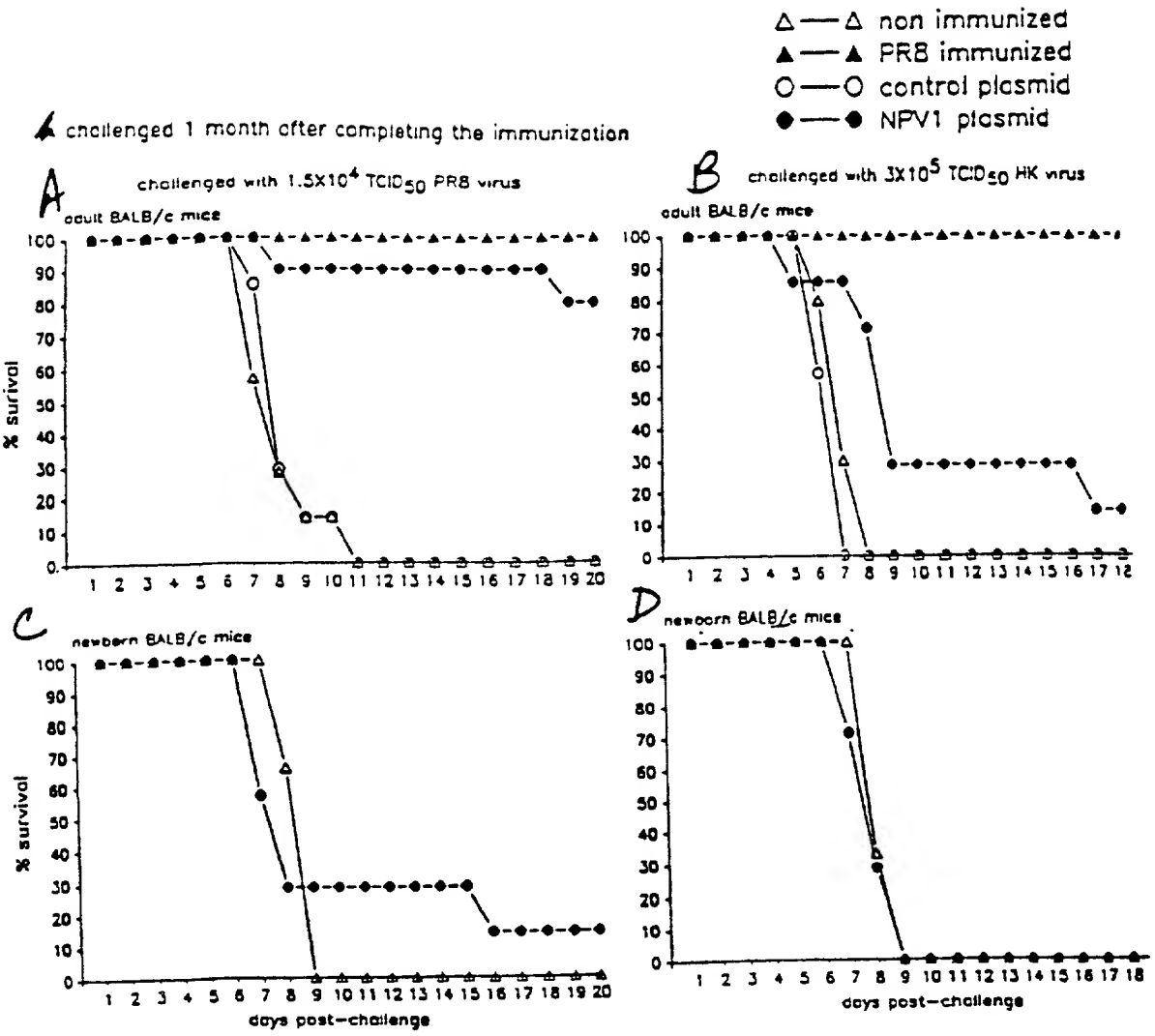
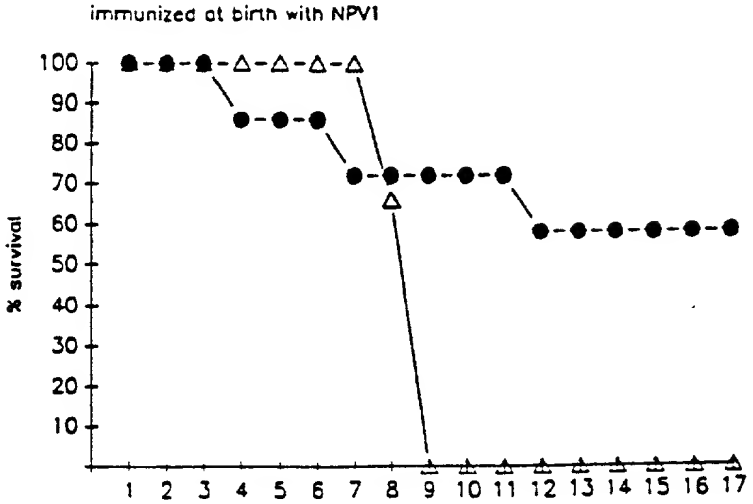

△—△ non immunized
▲—▲ PR8 immunized
○—○ control plasmid
●—● NPV1 plasmid
challenged 1 month after completing the immunization
A
challenged with 1.5X10^4 TCID50 PR8 virus
adult BALB/c mice
% survival
B
challenged with 3X10^5 TCID50 HK virus
adult BALB/c mice
C
newborn BALB/c mice
% survival
days post-challenge
D
newborn BALB/c mice
days post-challenge

Fig. 5A

E

challenged 3 months after completing immunization, with 1.5×10^4 $TCID_{50}$ PR8 virus



F

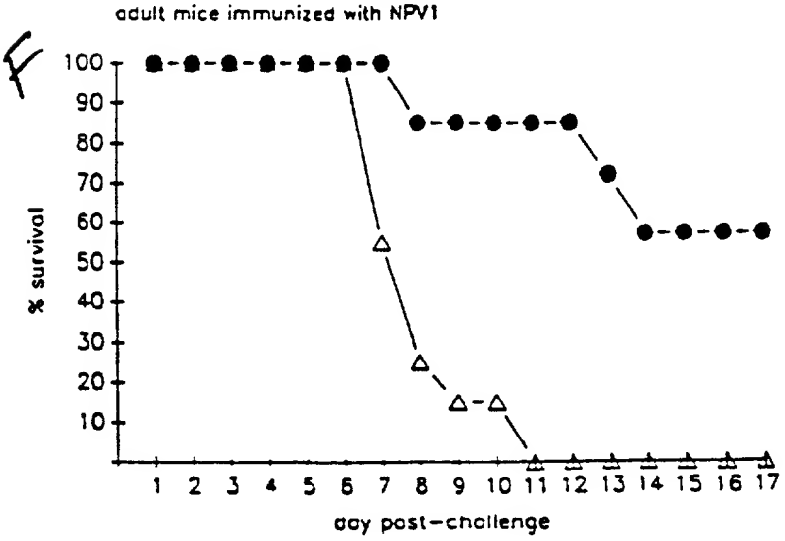


Fig. 5B

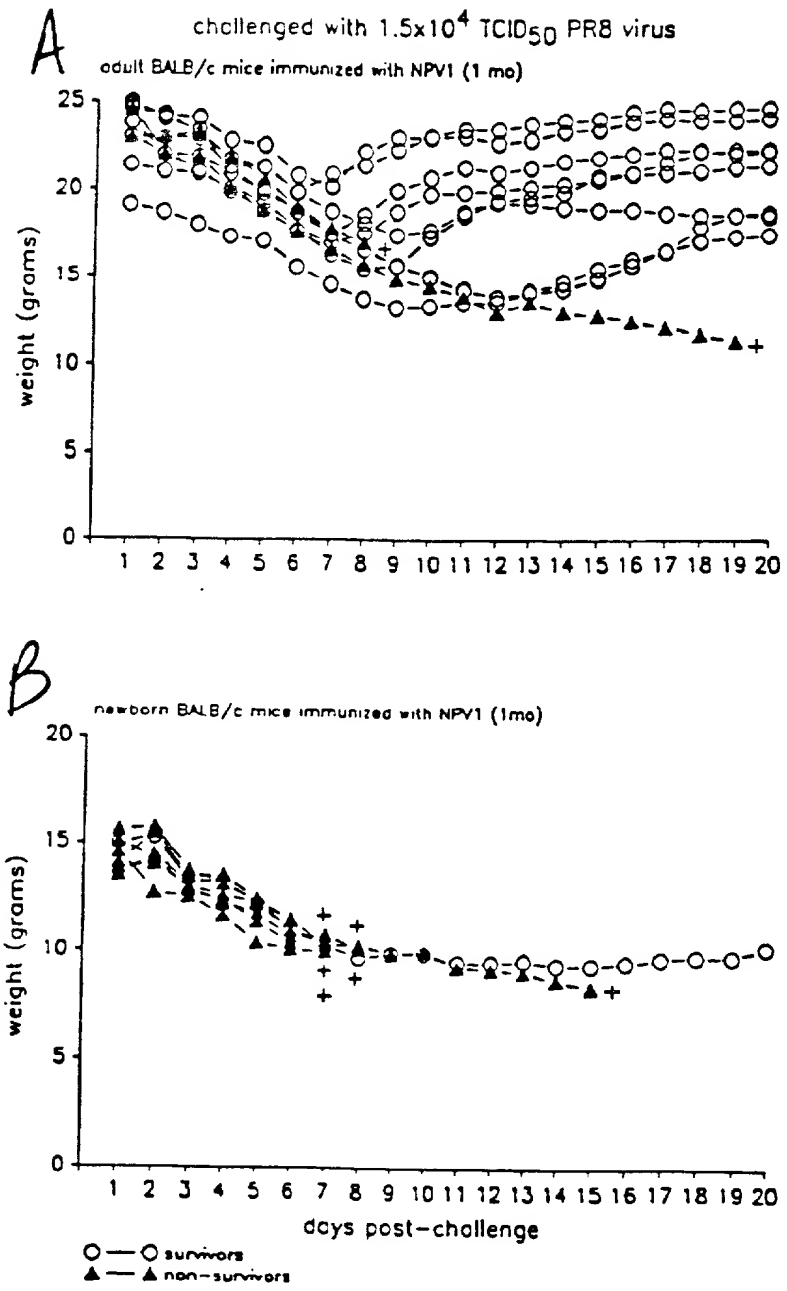

A
challenged with 1.5x10^4 TCID50 PR8 virus
adult BALB/c mice immunized with NPV1 (1 mo)
weight (grams)
B
newborn BALB/c mice immunized with NPV1 (1mo)
weight (grams)
days post-challenge
survivors
non-survivors

Fig. 6

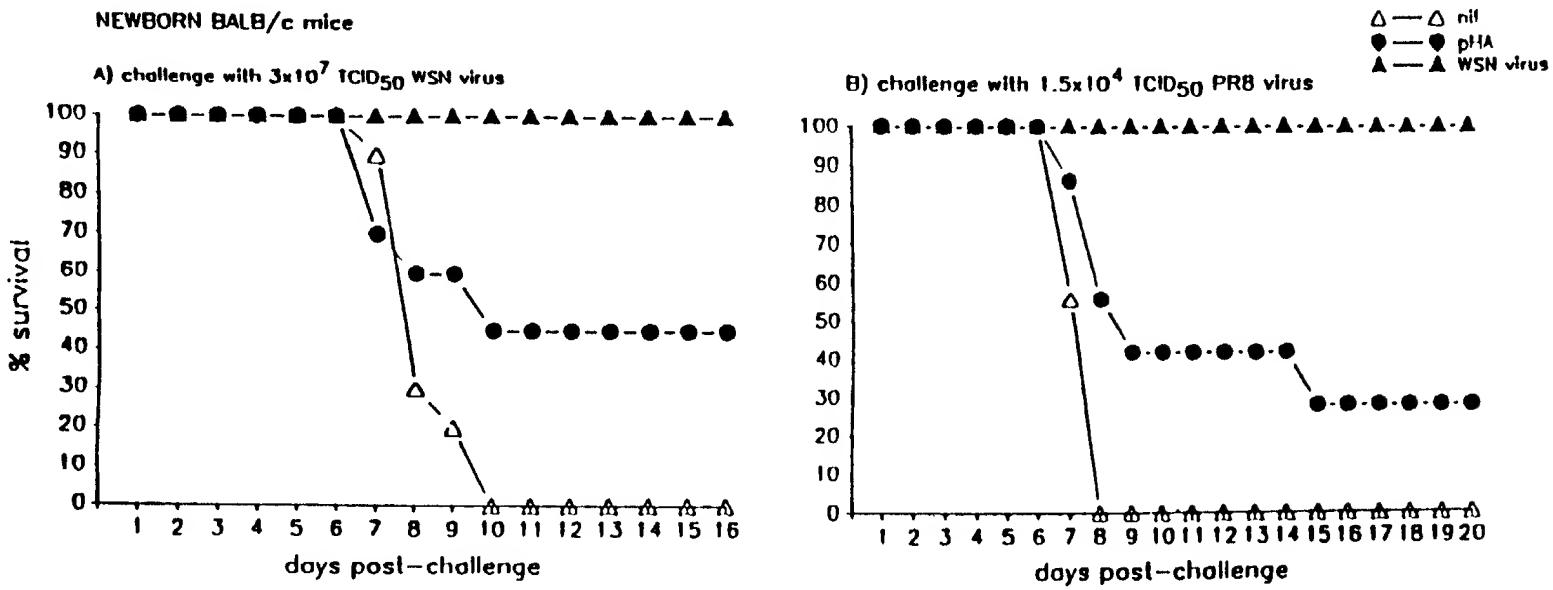

NEWBORN BALB/c mice
A) challenge with 3x10^7 TCID50 WSN virus
% survival
days post-challenge
B) challenge with 1.5x10^4 TCID50 PR8 virus
days post-challenge
nil
pHA
WSN virus

Fig. 7AB

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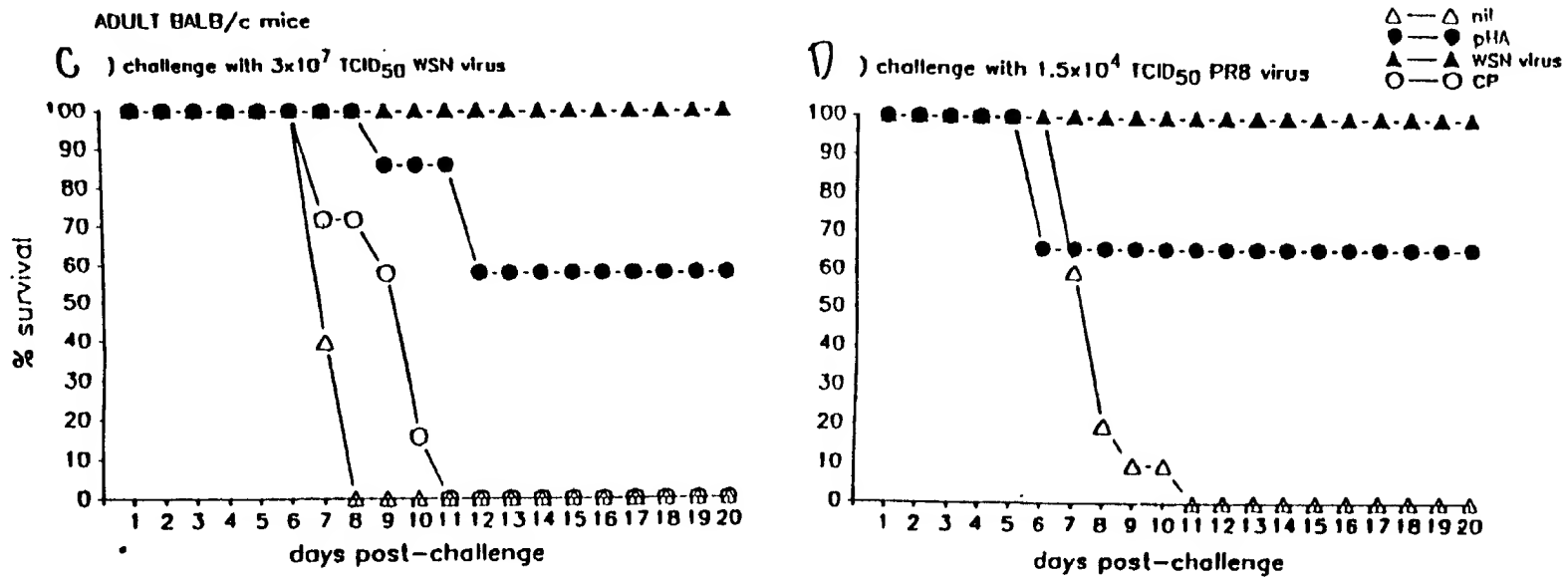
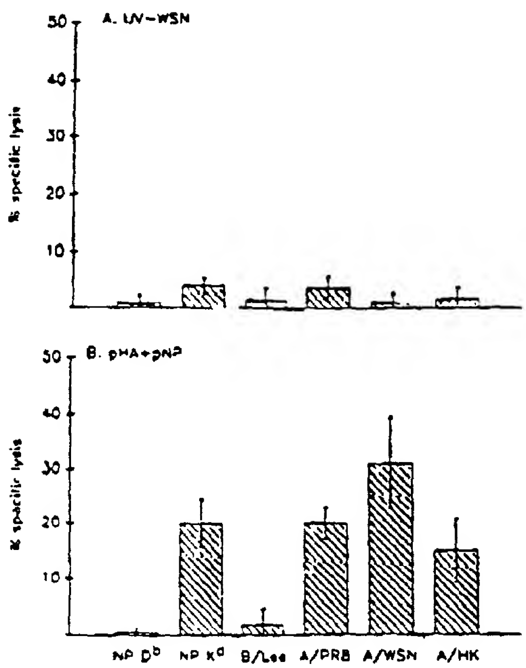


Fig. 7CD

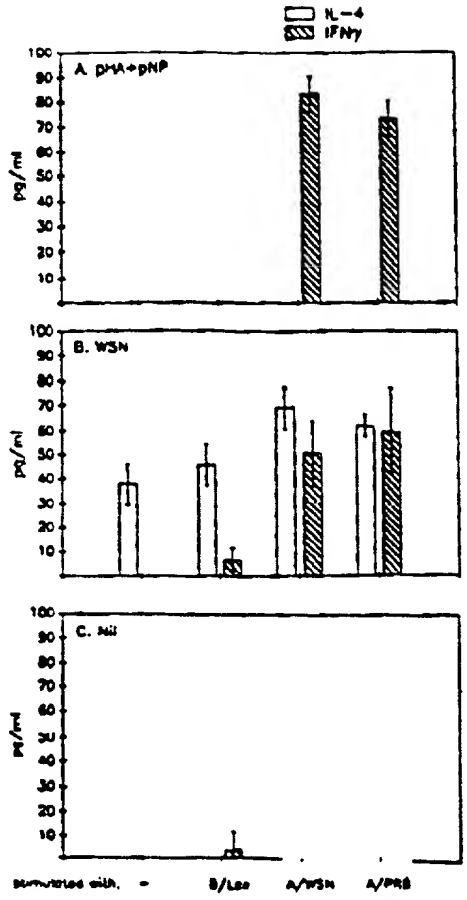
A

B



CTL response of mice immunized as newborns with UV-attenuated WSN virus (A) or a combination of pHA and pNP plasmids (B). Splenocytes pooled from three mice in each group were in vitro stimulated with PR8 virus-infected APC and tested against P815 cells coated with NP peptides or infected with various Influenza viruses, at E/T ratio of 10:1. The results are expressed as means of % specific lysis ± SD of triplicates.

Fig. 8



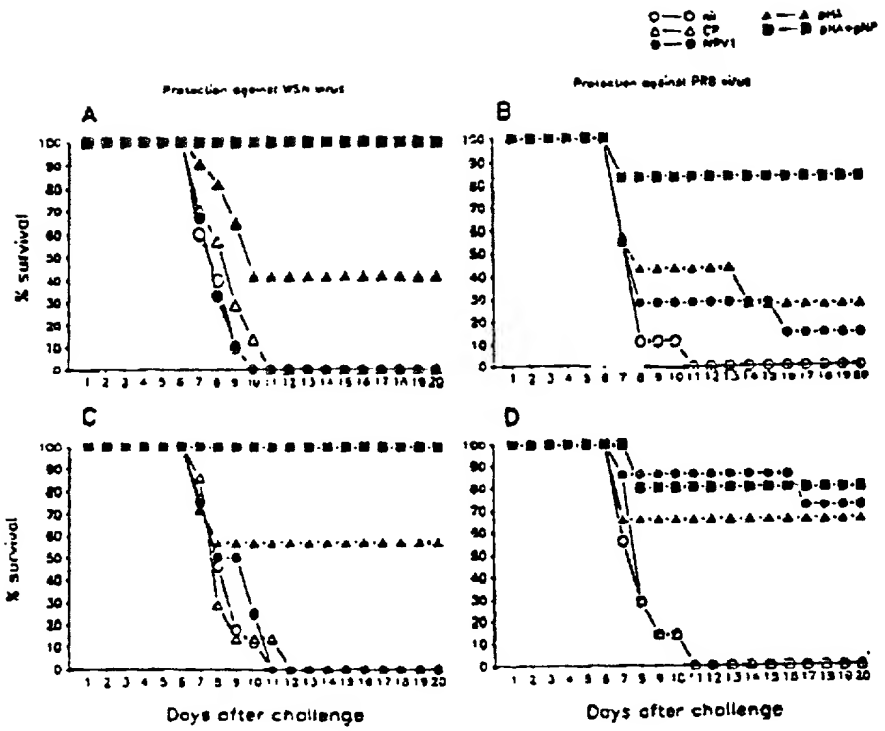
A

B

C

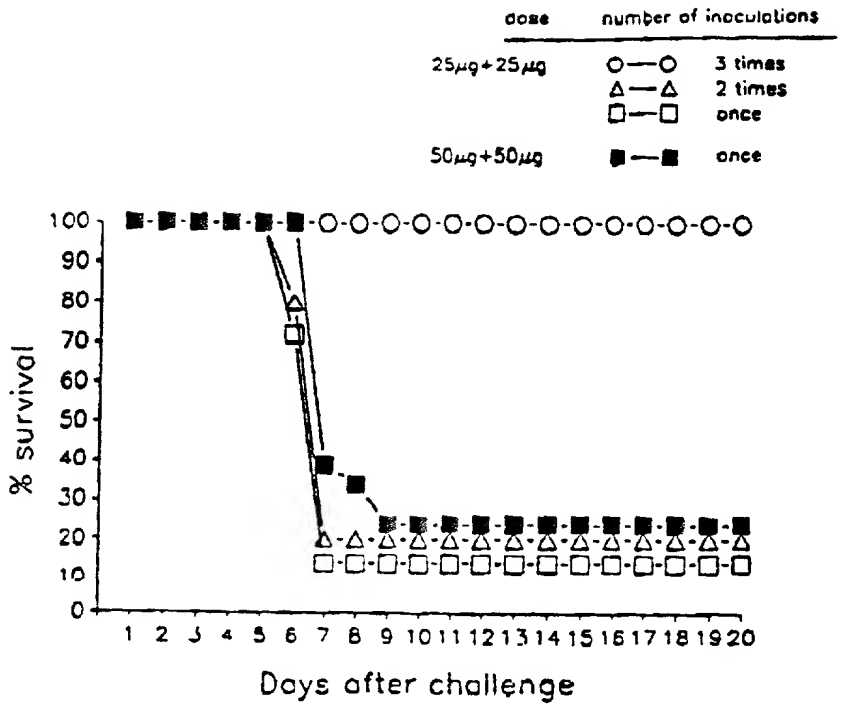
Cytokine secretion by $CD4^+$ T cells from mice immunized as neonates with a combination of pHA and pNP plasmids (A), UV-attenuated WSN virus (B) or nil (C). Negatively selected $CD4^+$ T cells were incubated four days in the presence of sucrose-purified UV-inactivated viruses (3µg/ml), APC and rIL-2 (6U/ml). The concentration of IFNγ and IL-4 was estimated by ELISA and the results were expressed as means of duplicates ± SD (pg/ml).

Fig 9



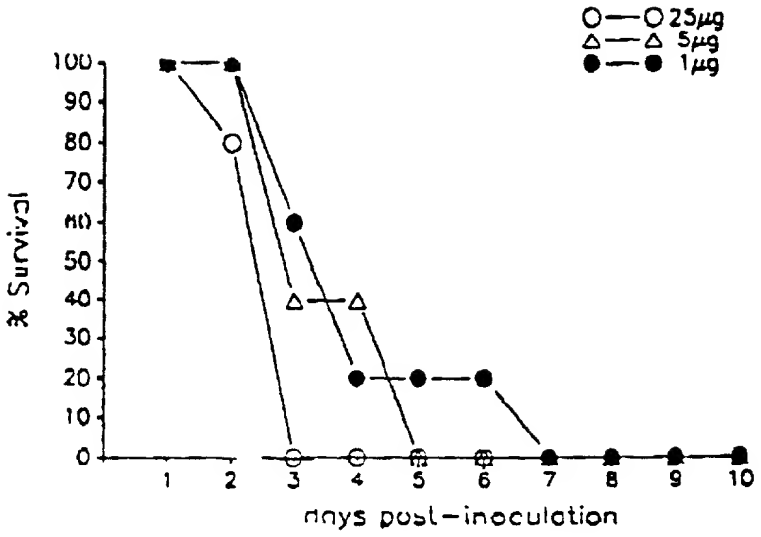
Protection against lethal challenge with WSN (A,C) or PR8 (B,D) virus of mice immunized as newborns (A,B) or adults (C,D) with a combination of pHA and pNP plasmids. As controls, we used naive mice, mice inoculated with a control plasmid (pRc/CMV) and mice immunized with pHA or pNP, separately. The mice were challenged with lethal doses of virus at four weeks following the completion of immunization.

Fig. 10



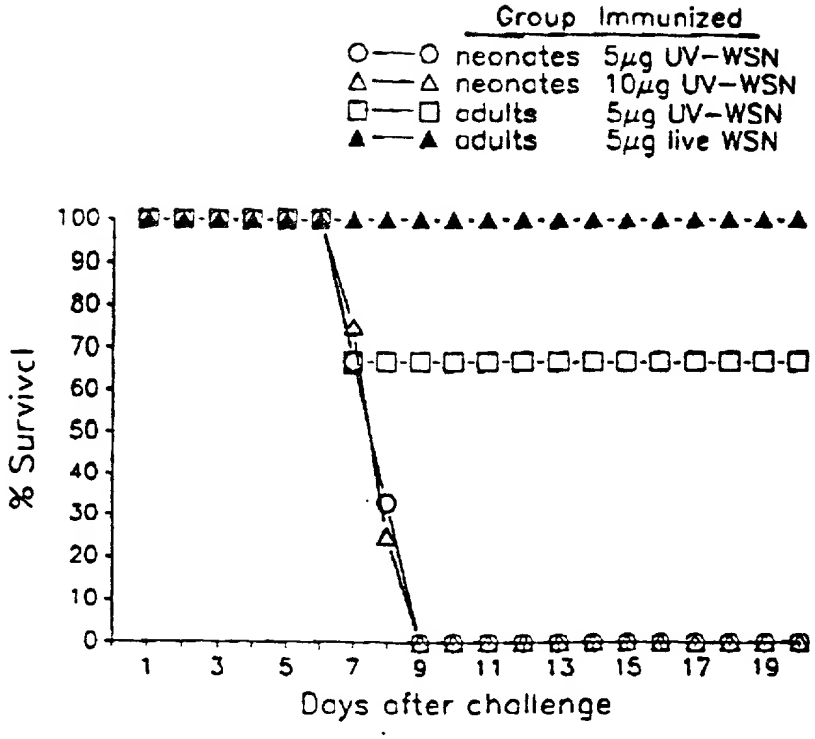
Dependence of the protection on the number of inoculations. The newborn mice were inoculated at day 1, 1 and 7, or 1, 3 and 6 with a mixture of pHA and pNP plasmids. At four weeks after the completion of immunization, the mice were challenged with a lethal dose of WSN virus.

Fig. 11



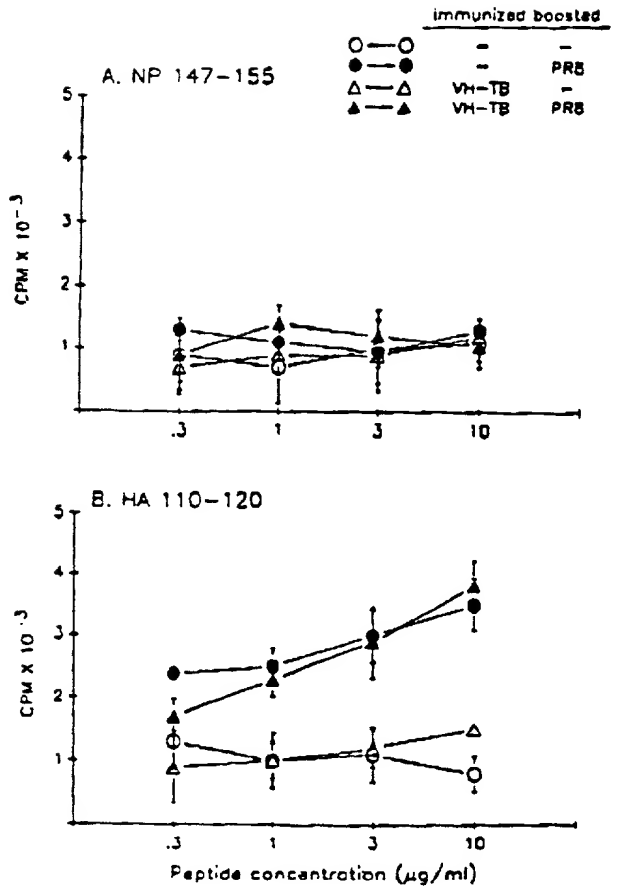
Lethality of live WSN virus following neonatal inoculation of mice. Various doses of live WSN virus were injected in the gluteal muscle of 1 day-old BALB/c mice. The survival of the neonates was followed for one week after the injection.

Fig. 12



Protection against the homologous challenge of mice immunized as adults or newborns with WSN virus. Mice were inoculated i.m. with UV-inactivated or live-WSN virus, in the case of adult mice and challenged four weeks later with a lethal dose of WSN virus.

Fig. 13



A

B

Proliferation of the CD4⁺ T cells from mice immunized as newborns with VH-TB plasmid. Negatively selected CD4⁺ T cells from mice immunized with VH-TB as neonates were incubated with APC in the presence of various concentrations of NP 147-155 (A) or HA 110-120 (B) synthetic peptides. ^{3}H-Thymidine was added after 72 hours and the radioactivity incorporated was measured after other 14 hours. The results are expressed as means of triplicates ± SD of proliferation indexes. Part of the mice immunized with VH-TB were boosted with PR8 virus. As controls, we used naive age-matched mice and mice immunized with live PR8 virus one week previous to the sacrification.

Fig. 14

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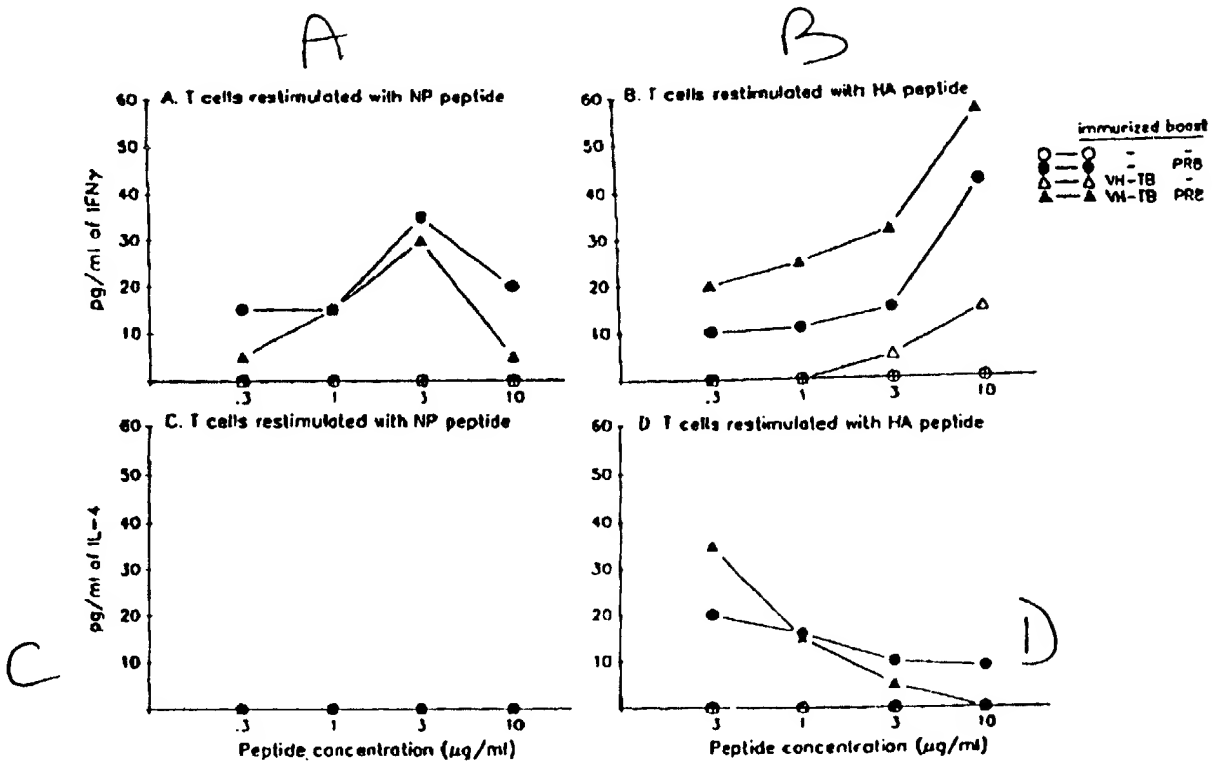
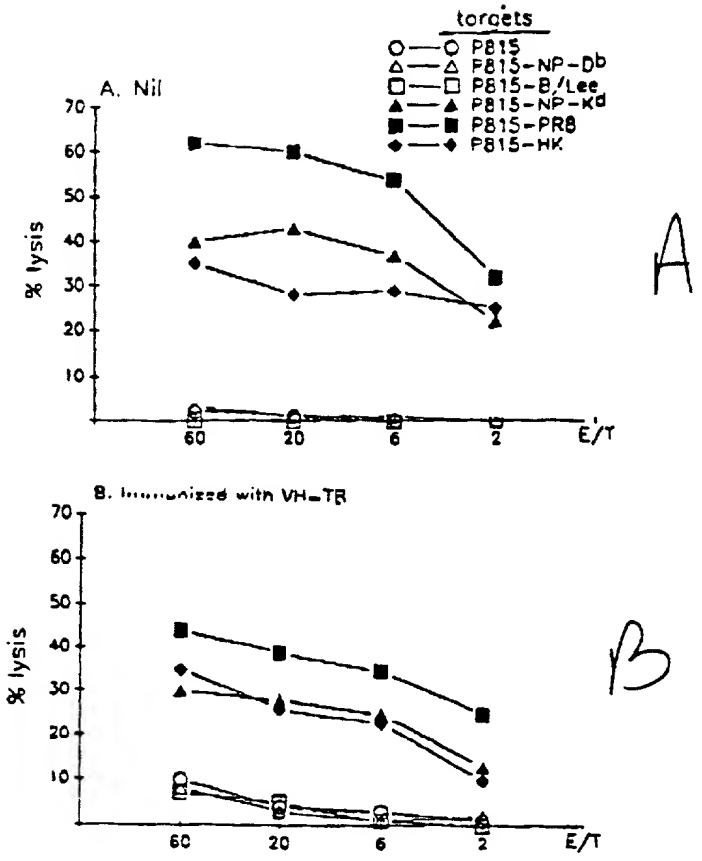


Cytokine production of the T cells from mice immunized as newborns with VH-TB plasmid Nylon wool-purified T cells from spleens of mice immunized as neonates with VH-TB were incubated with various concentrations of NP 147-155 (A,C) or HA 110-120 (B,D) synthetic peptides in the presence of APC and 6U/ml rIL-2. IFNγ (A,B) and IL-4 (C,D) were measured three days later by ELISA and the results were expressed as means of duplicates (pg/ml). SE was less than 25% of the mean, in each case. As controls, we used naive mice and mice immunized with PR8 virus one week previous to the sacrification. Part of the mice immunized with VH-TB were boosted with PR8 virus one week previous to the study.

FIG. 15

PCT/US92/04869



The CTL response to PR8 virus of mice immunized as neonates with VH-TB plasmid. Mice immunized with VH-TB as newborns were boosted three weeks later with live PR8 virus. The splenocytes from three mice in each group (injected only with PR8 virus - (A) and immunized with VH-TB and boosted with PR8 virus - (B)) were harvested and pooled one week later and in vitro stimulated with PR8 infected APC. The cytotoxicity was measured against P815 target cells infected with various strains of Influenza or coated with NP synthetic peptides. The results are expressed as means of % specific lysis of duplicates.

Fig. 16

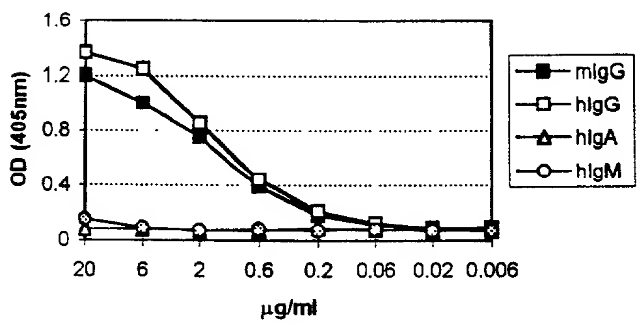
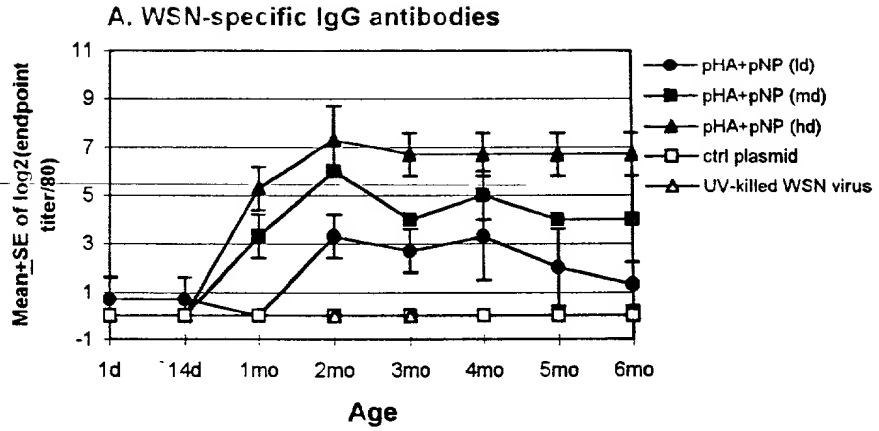

1.6
1.2
0.8
0.4
0
OD (405nm)
20
6
2
0.6
0.2
0.06
0.02
0.006
µg/ml
mIgG
hIgG
hIgA
hIgM

Figure 17


A. WSN-specific IgG antibodies
Mean+SE of log2(endpoint titer/80)
11
9
7
5
3
1
-1
1d
14d
1mo
2mo
3mo
4mo
5mo
6mo
Age
pHA+pNP (ld)
pHA+pNP (md)
pHA+pNP (hd)
ctrl plasmid
UV-killed WSN virus

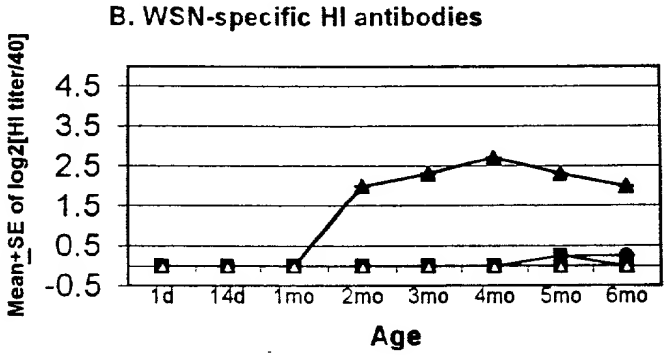
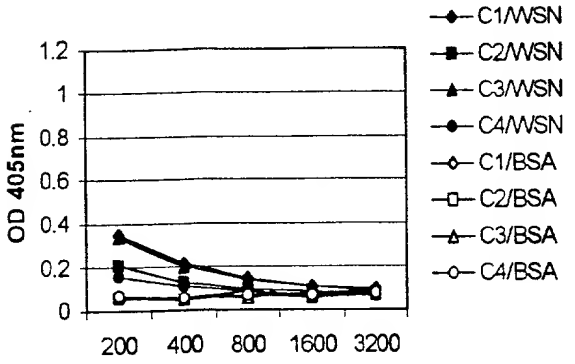
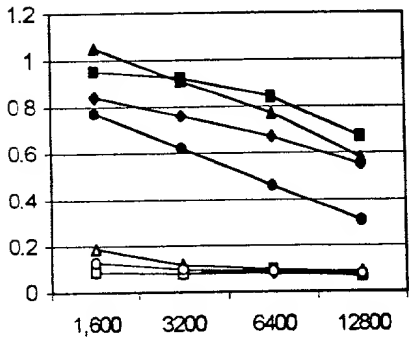

B. WSN-specific HI antibodies
Mean+SE of log2[HI titer/40]
4.5
3.5
2.5
1.5
0.5
-0.5
1d
14d
1mo
2mo
3mo
4mo
5mo
6mo
Age

Figure 1B

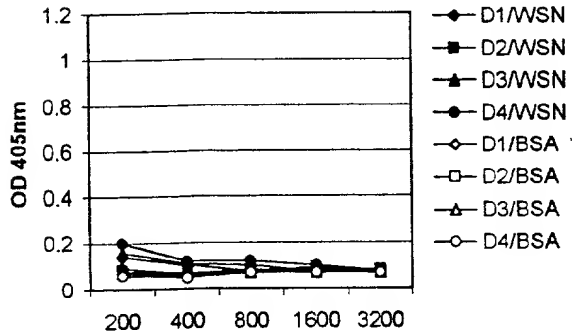
A. WSN-specific IgG before challenge
(pHA+pNP)



C. WSN-specific IgG after challenge
(pHA+pNP)



B. WSN-specific IgG before challenge
(Control Plasmid)



D. WSN-specific IgG after challenge
(Control Plasmid)*

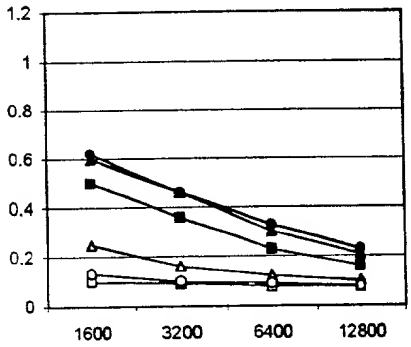


Figure 19

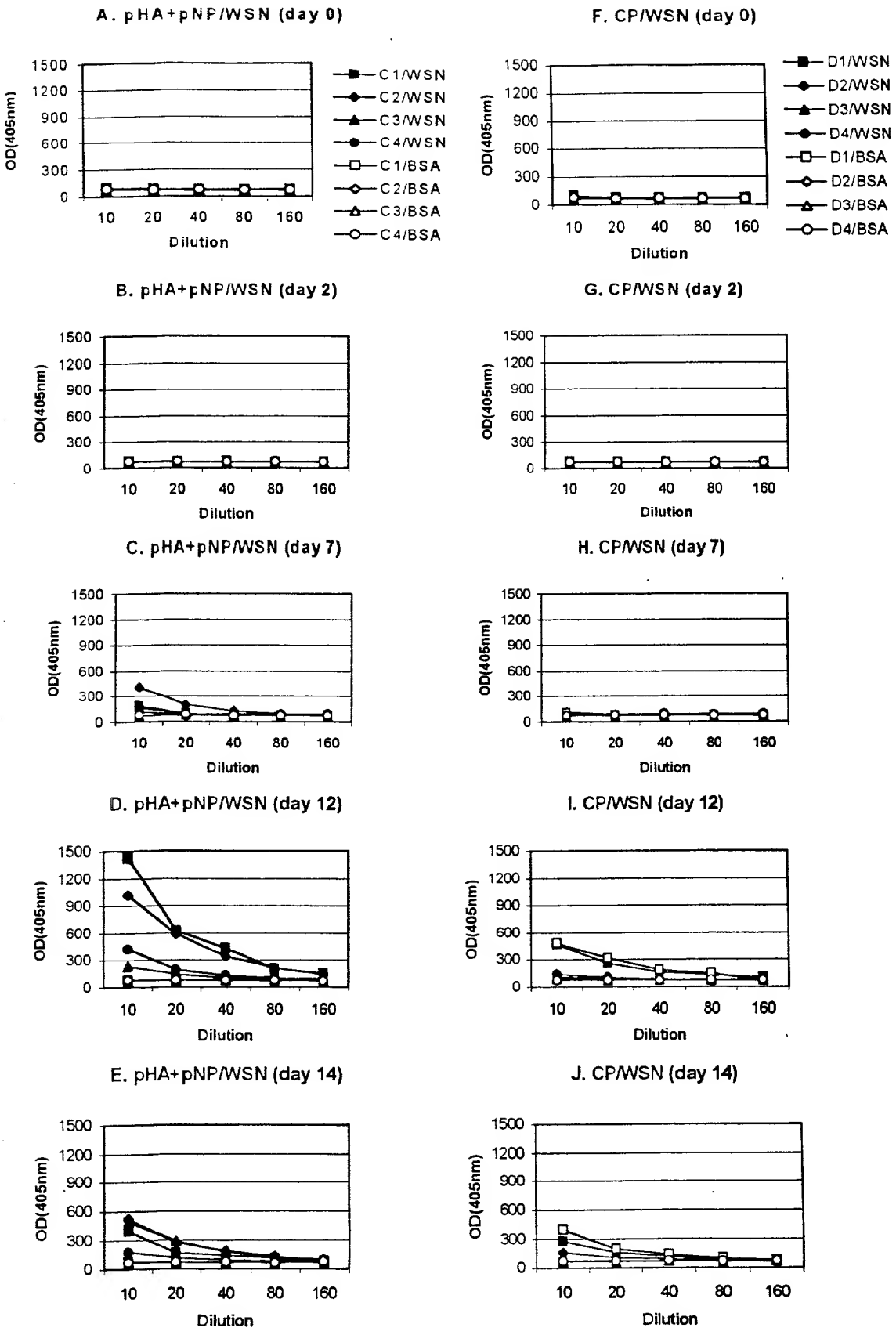

A. pHA+pNP/WSN (day 0)
F. CP/WSN (day 0)
B. pHA+pNP/WSN (day 2)
G. CP/WSN (day 2)
C. pHA+pNP/WSN (day 7)
H. CP/WSN (day 7)
D. pHA+pNP/WSN (day 12)
I. CP/WSN (day 12)
E. pHA+pNP/WSN (day 14)
J. CP/WSN (day 14)
OD(405nm)
Dilution
10
20
40
80
160
0
300
600
900
1200
1500
C1/WSN
C2/WSN
C3/WSN
C4/WSN
C1/BSA
C2/BSA
C3/BSA
C4/BSA
D1/WSN
D2/WSN
D3/WSN
D4/WSN
D1/BSA
D2/BSA
D3/BSA
D4/BSA

Figure 20

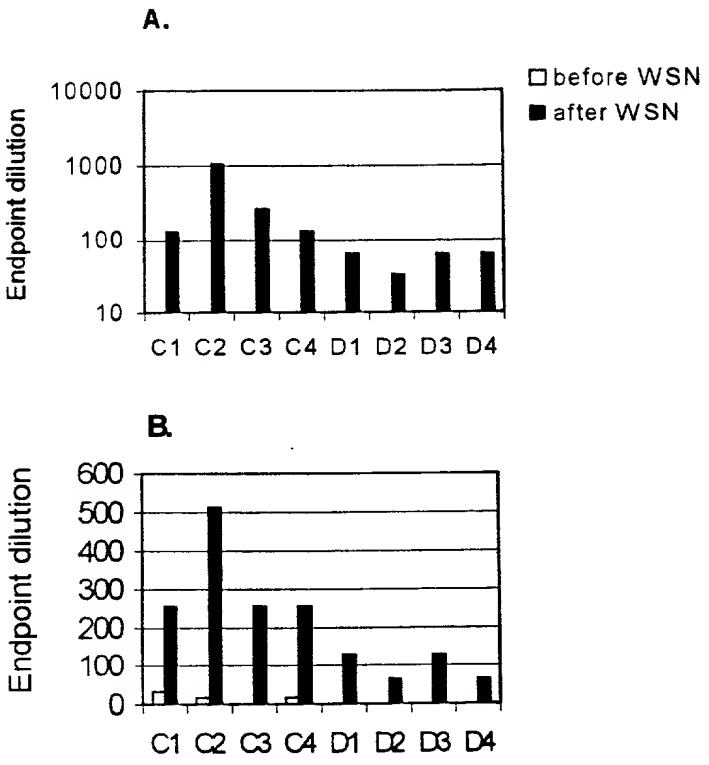

A.
before WSN
after WSN
Endpoint dilution
10000
1000
100
10
C1 C2 C3 C4 D1 D2 D3 D4
B.
Endpoint dilution
600
500
400
300
200
100
0
C1 C2 C3 C4 D1 D2 D3 D4

Figure 21